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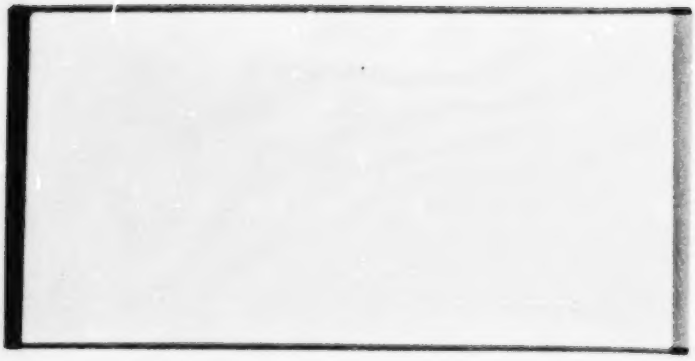

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Ontario Hydro, Research Division
800 Kipling Avenue,
Toronto, Ontario, Canada
M8Z 5S4

Telex: 06-984-525-706

Phone: (416)231-4111

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UPTAKE AND TRANSLOCATION OF 2,4-D, 2,4-DP, PICLORAM AND TRICLOPYR AFTER APPLICATION TO THE FOLIAGE OF CAROLINA POPLAR AND WHITE ASH

Report No 83-178-K

G.P. Arron
Biologist
Biological Research Section
Chemical Research Department

ABSTRACT

After application of the herbicides 2,4-D plus 2,4-DP, picloram plus 2,4-D and triclopyr to the foliage of Carolina poplar (Populus X canadensis Moench), a herbicide-sensitive woody species, and white ash (Fraxinus americana L.), a herbicide resistant woody species, leaves of white ash retained slightly more 2,4-D, 2,4-DP, picloram and triclopyr than leaves of Carolina poplar. A minimum of 30% of the 2,4-DP, 2,4-D (applied with 2,4-DP) and triclopyr retained by the leaves of both species one day after initial herbicide application appeared to have been taken up into the leaves. The amounts of 2,4-DP, 2,4-D (applied with 2,4-DP) and triclopyr found within the leaves of Carolina poplar and white ash declined by the third day after application to the former and by the seventeenth day after application to the latter. There appeared to be no decline in the amounts of picloram and 2,4-D (applied in combination) detected within the leaves of either species, up to eight days after application of the herbicides to Carolina poplar and up to seventeen days after the application to white ash. Some translocation of herbicide to the roots was observed as small amounts of triclopyr and 2,4-D were detected in the roots of Carolina poplar and a small amount of picloram was detected in white ash roots.

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EXECUTIVE SUMMARY

UPTAKE AND TRANSLOCATION OF 2,4-D, 2,4-DP, PICLORAM AND TRICLOPYR AFTER APPLICATION TO THE FOLIAGE OF CAROLINA POPLAR AND WHITE ASH

G.P. Arron
Biological Research Section
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Ontario Hydro employs the herbicides 2,4-D (2,4-dichlorophenoxy-acetic acid), 2,4-DP (2,4-dichlorophenoxypropionic acid), and picloram (4-amino-3,5,6-trichloropicolinic acid) to control brush and tree growth on rights-of-way. There have been reports of both woody and non-woody species which are resistant to one or more of these herbicides. It has been suggested that a basis for herbicide-resistance (after foliar-application of herbicides) is poor penetration of herbicide through the leaf cuticle and into the leaf cells. Herbicide-resistance may also be linked to a lack of movement of herbicide out of the treated leaf and into the root system. Effective control of woody plants is brought about by effective destruction of the whole root system.

In the present study, the uptake and translocation of 2,4-D, 2,4-DP, picloram and triclopyr {[(3,4,5-trichloro-2-pyridinyl)-oxy]acetic acid} (applied as a mixture of 2,4-D plus 2,4-DP, picloram plus 2,4-D or triclopyr alone) were determined at various times after foliar application to white ash (a herbicide-resistant woody species) and Carolina poplar (a herbicide-sensitive woody species).

One day after application of the herbicides to the foliage of Carolina poplar and white ash, leaves of the latter had retained slightly more 2,4-D, 2,4-DP, picloram and triclopyr either in or on the leaves, than leaves of the former, on a leaf fresh weight basis. Of the herbicide detected within or on the leaves of Carolina poplar and white ash, a minimum of 30% of the 2,4-DP,

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2,4-D (when applied with 2,4-DP) and triclopyr appeared to reside within the leaves one day after herbicide application. On the basis of herbicide detected per leaf, the amounts of 2,4-DP, 2,4-D (applied with 2,4-DP) and triclopyr within the leaves of Carolina poplar and white ash declined by the third day after application to the former species and by the seventeenth day after application to the latter. There was no decline (on a weight of herbicide detected per leaf basis) in the amount of picloram or 2,4-D (applied with picloram) found within the leaves of Carolina poplar up to eight days after herbicide application or within the leaves of white ash up to seventeen days after herbicide application. There was little translocation of any of the applied herbicides from the foliage of either species to the roots, since only small amounts of triclopyr and 2,4-D (applied with 2,4-DP) were detected in Carolina poplar root tissue, and a small amount of picloram was the only herbicide detected in white ash root tissue, both on a fresh weight basis.

There was no correlation between either a lack of retention of any of the applied herbicides on foliage, or a lack of uptake of the retained herbicide into the leaf, and the overall herbicide-resistance of white ash. Although both triclopyr and 2,4-D were detected in the roots of Carolina poplar, neither herbicide was detected in the roots of white ash.

TABLE OF CONTENTS

		Page
1.0	INTRODUCTION	1
2.0	MATERIALS AND METHODS	3
2.1	Plant Material	3
2.2	Herbicide Application	3
2.3	Cross-Contamination of Trees After Herbicide Application	4
2.4	Collection and Initial Preparation of Plant Tissue Samples	4
2.5	Herbicide Extraction	4
2.6	Herbicide Analysis	5
2.6.1	Methodology	5
2.6.2	Detection Limits in Analysis	5
3.0	RESULTS AND DISCUSSION	6
3.1	Herbicides Detected in Leaves of Carolina Poplar and White Ash Trees After Application of the Herbicides 2,4-D plus 2,4-DP, Picloram Plus 2,4-D and Triclopyr	6
3.2	Translocation of the Herbicides 2,4-D, 2,4-DP, Picloram and Triclopyr to the Roots of Carolina Poplar and White Ash After Application of 2,4-D Plus 2,4-DP, Picloram Plus 2,4-D or Triclopyr to the Foliage	16
4.0	CONCLUSIONS	20
5.0	REFERENCES	23
APPENDICES		24
DISTRIBUTION		last page

LIST OF TABLES

		Page
Table I	Herbicide Detected In 'Unwashed' Carolina Poplar Leaves Sprayed with 2,4-D Plus 2,4-DP or Picloram Plus 2,4-D or Triclopyr	7
Table II	Herbicide Detected in 'Unwashed' White Ash Leaves Sprayed with 2,4-D Plus 2,4-DP or Picloram Plus 2,4-D or Triclopyr	9
Table III	Herbicide Detected in 'Washed' Carolina Poplar Leaves Sprayed with 2,4-D Plus 2,4-DP or Picloram Plus 2,4-D or Triclopyr	11
Table IV	Herbicide Detected in 'Washed' White Ash Leaves Sprayed with 2,4-D Plus 2,4-DP or Picloram Plus 2,4-D or Triclopyr	12
Table V	Proportion of Herbicide Detected in 'Washed' Leaves of Carolina Poplar and White Ash to Herbicide Detected in 'Unwashed' Leaves, After Application of 2,4-D Plus 2,4-DP or Picloram Plus 2,4-D or Triclopyr	14
Table VI	Herbicide Detected in Carolina Poplar Roots Nine Days After Application to the Foliage of 2,4-D Plus 2,4-DP or Triclopyr	17
Table VII	Partial List of Reports on the Herbicide Content of Roots of Woody Species in Which Triclopyr, Picloram or 2,4,5-T Had Been Applied to the Foliage	19

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To Mr. F.J. Kee
Director of Research

UPTAKE AND TRANSLOCATION OF 2,4-D, 2,4-DP, PICLORAM AND TRICLOPYR AFTER APPLICATION TO THE FOLIAGE OF CAROLINA POPLAR AND WHITE ASH

1.0 INTRODUCTION

Ontario Hydro employs the herbicides 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4-DP (2,4-dichlorophenoxypropionic acid), and picloram (4-amino-3,5,6-trichloropicolinic acid) to control brush and tree growth on rights-of-way. These herbicides are applied as a mixture of 2,4-D and 2,4-DP, or a mixture of picloram and 2,4-D, in the form of a foliar and stem spray, a direct application of the herbicide mixture to areas of cut bark around the base of the tree (basal treatment) or to the cut stump. Alternatively, herbicide pellets are distributed on the ground around the problem plant (root presentation).

The effective control of woody plants with herbicides depends on several factors, among the most important of which are the woody species involved, herbicide used, herbicidal rate, formulation, method of application, and various ecological phenomena. There have been reports of both woody and non-woody species which are resistant to one or more of the herbicides 2,4-D; 2,4-DP; picloram and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid: use of this herbicide in Ontario was banned in 1979). In many instances it has not been possible to determine the biological basis of such herbicide resistance. In the case of foliar-applied herbicides, it has been suggested that resistance may be linked to a lack of penetration of herbicide through the cuticle and into the leaf cells. Davis et al (1968b) reported the poor penetration of 2,4,5-T and picloram into the leaves of live oak (Quercus virginiana Mill), where only 1% and 2% respectively, of the applied herbicide was absorbed after 48 hours. However, Eliasson and Hallmen (1973) found that 44% of the picloram and 57% of the 2,4-D applied to aspen (Populus tremula) had entered the leaves three days after application. The obvious inconsistencies both between these and other reports may be explained by

differences in the methods used to remove herbicide not taken up into the leaf cells and the fact that different tree species may take up different amounts of the herbicide applied to the foliage.

Herbicide resistance may also be linked to a lack of movement of herbicide out of the treated leaf, even when herbicide has entered the individual leaf cells efficiently. Davis et al (1968c) found that 99% of the picloram and 97% of the 2,4,5-T taken up by leaves of velvet mesquite (Prosopis juliflora) remained in the cells of treated leaves 90 hours after application.

Of the herbicide applied to the foliage of a plant, only small quantities appear to be translocated to the roots. Mitchell and Stephenson (1973) reported that of the ^{14}C-picloram applied to a mature leaf of either white ash (Fraxinus americana L.) (herbicide-resistant species) or red maple (Acer rubrum L.) (herbicide-sensitive species) 97% in both cases remained in or on the leaf, and 1.18% and 0.81% respectively, reached the roots of the tree after 12 days. Thus there did not appear to be any relationship between herbicide tolerance and an inability of applied herbicide to reach the roots after foliar application. Since the concentration of picloram was also slightly higher in the white ash roots, it was concluded that the roots (and presumably the leaves as well) of tree species differ in their susceptibility to the toxic effects of herbicides.

Triclopyr has been reported to effectively control certain woody plants and perennial weeds (Byrd et al, 1977; Thomson, 1977). Bovey and Mayeux (1980) found triclopyr to be as effective as 2,4,5-T and picloram for control of honey mesquite (P. juliflora var. glandulosa) and that triclopyr concentrations in root tissue were comparable to those of 2,4,5-T and picloram after foliar-application of the herbicides.

In the present study, the uptake and translocation of 2,4-D; 2,4-DP; picloram and triclopyr (applied as a mixture of 2,4-D and 2,4-DP; a mixture of picloram and 2,4-D; and triclopyr alone) were determined at various times after foliar-application to white ash (Fraxinus americana L.) (a herbicide-resistant species) and Carolina poplar (Populus X canadensis Moench) (a herbicide-sensitive species). This information will provide an insight into the basis of herbicide-resistance in white ash, whether it be lack of retention of herbicide on the leaf, poor penetration into the leaf, or inefficient movement to the roots. The herbicides were applied to the tree material in a manner as similar as possible to that used by Ontario Hydro on rights-of-way (similar herbicide concentration, volume of spray applied, and method of application). The effects of these

herbicides on various aspects of white ash and Carolina poplar metabolism (eg, leaf transpiration, photosynthesis, leaf and root respiration) have been described previously (Ontario Hydro Research Division Report No 83-88-K).

2.0 MATERIALS AND METHODS

2.1 Plant Material

Two-year old white ash (Fraxinus americana L.) and Carolina poplar (Populus X canadensis Moench) trees were a gift from the Ontario Ministry of Natural Resources (collected from the Midhurst Station). The former were about 0.5 m in height and the latter, about 2 m. The white ash trees were planted in 30 cm pots in a one-to-one mixture of shredded topsoil (native sandy loam) and peat moss (Plant Products, Bramalea, Ontario) and the Carolina poplar trees were planted in 37 cm pots in the same mixture. The trees were placed in the greenhouse immediately after potting and watered at regular intervals. At weekly intervals the topsoil in each pot was dug over and fertilizer applied (solution of 20-20-20 (NPK) obtained from Plant Products). Approximately one to two weeks after planting 95% of both the white ash and Carolina poplar trees had broken dormancy. Herbicides were applied to the trees (see below) five to six weeks after breakage of dormancy. Forty-eight trees of each species were used for each herbicide treatment, and forty-eight for the control.

2.2 Herbicide Application

The herbicides picloram plus 2,4-D; and triclopyr were obtained from Dow Chemical Company, Sarnia, Ontario; and the mixture of 2,4-D and 2,4-DP was obtained from Union Carbide, Chatham, Ontario.

For a detailed description (full chemical name, concentration) of the herbicides used, see Appendix A.

The stock mixture of 2,4-D and 2,4-DP, and the stock mixture of picloram and 2,4-D were both diluted 100 times with tap water to give a 1% solution, before application to the trees. Triclopyr was diluted 133 times to give a 0.75% solution. These dilutions are those recommended by the herbicide manufacturers.

The herbicides were applied to the tree foliage (to leaf drip) using a small hand sprayer at 25 psi with a Tee Jet 8004 nozzle. The pots were covered with plastic sheets to prevent excess herbicide dripping onto the soil surface. After spraying, the trees were returned to one compartment of the green-

house; unsprayed (control) trees were located in the other compartment.

2.3 Cross-Contamination of Trees After Herbicide Application

Since all the treated trees (regardless of herbicide applied) were kept in the same compartment of the greenhouse, it was possible that cross-contamination of foliage might occur because of the volatility of the various herbicides. On the basis of the herbicide detection results obtained, it seems that such cross-contamination occurred infrequently, in that only triclopyr was found on leaves not treated with the herbicide, and then only on one sampling day.

2.4 Collection and Initial Preparation of Plant Tissue Samples

At various times after application of herbicide to the trees, samples of foliage and roots (both in triplicate) were collected for subsequent herbicide analysis. Root samples were obtained by removing the tree from the soil and then carefully brushing away the soil around the roots. The roots were washed in a bucket of water to remove the remaining soil particles, drained, weighed and placed in a plastic bag. Foliage samples (six to fifteen leaves, collected at random from four or more trees) were weighed and placed in plastic bags.

Leaves which were not washed prior to weighing are referred to as 'unwashed'. 'Washed' leaves were rinsed with a 0.1% NH_4OH aqueous solution (1% ethanol was added to lower surface tension) to remove herbicide remaining on the leaf surface, dried, and then weighed.

Samples of leaves and roots were stored at -16°C prior to herbicide extraction.

White ash has compound leaves, and although in the text reference will be made to white ash leaves, strictly speaking they should be termed leaflets.

2.5 Herbicide Extraction

Thawed leaf and root tissue (approximately 5 to 15 g) was cut into 2 cm pieces and homogenized with 150 mL of acidified (1:1000 12N HCl:acetone, v/v) acetone in a Virtis or Polytron blender. Residual fibrous plant material was removed by filtration (Whatman No 1) and 20 mL of 1% NH_4OH was added and the acetone was evaporated. The remaining basic solution was brought to 30 mL (with distilled H_2O) and washed with 100 mL of diethyl ether (pesticide analysis grade). The clear aqueous portion was drained from the separatory funnel into a beaker. A second

30 mL aliquot of 0.1% NH_4OH was added to the ether to remove additional herbicide. After separation the aqueous layer was drained from the separatory funnel and combined with the first extract. The top ether layer was discarded. The combined aqueous portion was acidified (pH 2) by dropwise addition of 6N HCl and extracted with four, 20 mL aliquots of diethyl ether. The four ether fractions were combined and evaporated down to a volume of approximately 5 mL. These samples were then sent to Analytic Services for herbicide analysis.

2.6 Herbicide Analysis

2.6.1 Methodology

Ether extracts were evaporated to dryness, taken up in acetonitrile and the herbicide content determined by HPLC (high-pressure liquid chromatography). The herbicide content of the extract was then expressed as a function of a) the number of leaves in the initial sample, or b) the fresh weight of the initial sample. In the case of root samples, results were expressed as a function of fresh weight only.

Initial experiments in which leaf and root material collected from trees not sprayed with herbicide, was "spiked" with a known amount of herbicide (2,4-D; 2,4-DP; picloram and triclopyr), and the herbicides then extracted and estimated by the procedures described above, indicated that retrieval rates of between 80 and 90% were obtained for all the herbicides with the exception of 2,4-DP added to leaf material where recovery was variable. Attempts to discover the cause of this variability were unsuccessful. The 2,4-DP did not appear to remain in either the initial ether fraction during washing of the aqueous NH_4OH fraction (this ether fraction was discarded normally) or the acidified aqueous fraction (also discarded after removal of the herbicide with ether).

2.6.2 Detection Limits in Analysis

For all 'unwashed' leaf samples the detection limit in the ether extract was 50 µg of each herbicide, and for all 'washed' leaf and root samples it was 10 µg. This difference was because a more sensitive detection apparatus was acquired by Analytic Services halfway through the experiment. Since the number and fresh weight of the leaves (and the fresh weight of the root samples) varied from sample to sample, the detection limit was thus not consistent between samples. In general the detection limit for 'unwashed' leaves was approximately 10 µg herbicide per leaf or per g leaf fresh weight, and for 'washed' leaves and roots it was approximately 2 µg herbicide per leaf or per g root or leaf fresh weight.

3.0 RESULTS AND DISCUSSION

3.1 Herbicides Detected in Leaves of Carolina Poplar and White Ash Trees After Application of the Herbicides 2,4-D Plus 2,4-DP, Picloram Plus 2,4-D and Triclopyr

The herbicides detected in extracts prepared from Carolina poplar leaves which had received one of the various herbicide treatments (2,4-D plus 2,4-DP or picloram plus 2,4-D or triclopyr) are given in Table I. These leaves were not washed with NH_4OH prior to weighing, and thus herbicide was present on the leaf surface, and within the leaf. The results given in Table I are expressed on a µg herbicide per leaf basis, because it was found that after application of the herbicides, the leaves became dehydrated and to relate herbicide content to fresh weight led to misleading results. In Carolina poplar leaves sprayed with 2,4-D plus 2,4-DP, the amount of 2,4-D apparently increased from 236 ± 26 µg/g fresh weight at day plus one, to 531 ± 83 µg/g fresh weight at day plus eight. By seven days after application of each of the herbicide treatments to Carolina poplar, most of the leaves were discoloured, and by 15 days all the leaves were dead (Ontario Hydro Research Division Report No 83-88-K). Discolouration and foliar death in white ash took slightly longer. Many previous reports on the uptake of herbicide into foliage have presented data on a per g fresh weight basis only (eg, Bovey and Mayeux, 1980) and for this reason the present data are expressed in both ways (see Appendix B).

The amount of 2,4-D and 2,4-DP detected in 'unwashed' leaves of Carolina poplar sprayed with 2,4-D plus 2,4-DP changed little up to eight days after herbicide application (Table I). Although the mixture of 2,4-D plus 2,4-DP applied, contained equal amounts of each herbicide, the amount of 2,4-DP detected in leaf samples was consistently less than the amount of 2,4-D detected. Given the problems encountered during retrieval of 2,4-DP added to foliage immediately before extraction (described more fully in Materials and Methods) and the lack of previous reports on the retrieval from foliage of 2,4-DP relative to 2,4-D (after application of an equimolar mixture of the two herbicides) this disproportionate recovery should be interpreted cautiously. At day three, foliage samples from trees sprayed with 2,4-D plus 2,4-DP were found to contain triclopyr, and this was presumably the result of cross-contamination in the greenhouse (discussed in Materials and Methods). No other cases of cross-contamination were encountered in the present study.

In 'unwashed' leaves of Carolina poplar sprayed with picloram plus 2,4-D in a one to four ratio by weight, both herbicides were detected, but the ratio was consistently more than one to four (see Table I). The amount of picloram and 2,4-D detected

TABLE I

HERBICIDE DETECTED IN 'UNWASHED' CAROLINA POPLAR LEAVES
SPRAYED WITH 2,4-D PLUS 2,4-DP OR PICLORAM PLUS 2,4-D OR TRICLOPYR

Herbicides were extracted from leaf material and analyzed as described in Materials and Methods. Three extracts were prepared for each treatment at each time interval and the results given constitute the mean ± one standard deviation. Full results are given in Appendix B

Herbicide Applied	Herbicide Detected	Days After Herbicide Applied				
		+1	+2	+3	+8	+14
		µg Herbicide Per Leaf				
2,4-D plus 2,4-DP	2,4-D	210±23	162±23	141±19	164±28*	-
	2,4-DP	88±11	49±7	20±7	53±10*	-
	Picloram	nd	nd	nd	nd	-
	Triclopyr	nd	nd	(26±10)†	nd	-
Picloram plus 2,4-D	2,4-D	167±18	192±4	165±16	254±40	268±68
	2,4-DP	nd	nd	nd	nd	nd
	Picloram	74±3	69±2	65±7	130±14	111±53
	Triclopyr	nd	nd	34±11	nd	nd
Triclopyr	2,4-D	nd	nd	nd	nd	nd
	2,4-DP	nd	nd	nd	nd	nd
	Picloram	nd	nd	nd	nd	nd
	Triclopyr	181±35	174±25	100±9	133±37	137±51

nd = not detected; * = 2 samples analyzed; - = no samples;

† = Mean of two (out of three) samples, probably contaminated in the greenhouse - see discussion on cross-contamination in Materials and Methods.

appeared to go up with time. Since such increases are impossible, this means that small-scale fluctuations in the data, either up or down, may result from errors in the whole analysis procedure and as such should be ignored or treated with extreme caution. The amount of triclopyr detected in 'unwashed' Carolina poplar leaves sprayed with the herbicide, did not change up to fourteen days after treatment (bottom of Table I). The lack of any decline in the amount of 2,4-D recovered from Carolina poplar leaves is in conflict with earlier studies on the disappearance of ^{14}C-labelled 2,4-D applied to aspen (Populus tremula L) leaves (Eliasson and Hallmen, 1973) where 76% of the applied label was present after one day, and 57% remained after nine days. The same authors found that picloram levels also changed little with time, with 74% of applied ^{14}C-picloram being found after one day, and 61% after nine days. Bovey and Mayeux (1980) reported that the amount of picloram and triclopyr in and on the foliage of honey mesquite (Prosopis juliflora var glandulosa) did not decline from day three to day ten after herbicide application. In fact the amount of picloram expressed on a fresh weight basis appeared to go up (from 375 to 436 µg picloram per g leaf fresh weight) and it may be that by day ten the foliage had been become partially dehydrated, however this was not addressed by the authors. In recent studies on triclopyr persistence in foliage on a right-of-way, only 50% of the applied herbicide was detected one day after spraying, 10% after fourteen days and 1% after 56 days (J. Jotcham personal communication). Such a rapid disappearance of applied herbicide might be the result of rapid translocation out of the leaves, volatilization or photolytic breakdown. Such a report of the rapid disappearance of triclopyr remains atypical however.

The herbicides detected in 'unwashed' white ash leaves sprayed with the various herbicide treatments are given in Table II. The amounts of 2,4-D and 2,4-DP in leaves sprayed with that mixture did not change consistently with time. Again the amounts of 2,4-DP detected were less than the amounts of 2,4-D, but with one exception. In leaves sprayed with picloram plus 2,4-D or triclopyr there was a drop in the amount of 2,4-D retrieved by fourteen days after herbicide application.

In a comparison of the amount of each herbicide detected in 'unwashed' leaves of Carolina poplar and white ash assayed one day after herbicide application, on a fresh weight basis (see Appendices B and C) the white ash leaves have slightly more of each herbicide either on the surface or within the leaf, than Carolina poplar leaves. Thus herbicide retention after foliar-application of all the herbicides used appears to be greater in the herbicide-resistant species, rather than as might be expected in the herbicide-sensitive species.

TABLE II

HERBICIDE DETECTED IN 'UNWASHED' WHITE ASH LEAVES SPRAYED WITH 2,4-D PLUS 2,4-DP OR PICLORAM PLUS 2,4-D OR TRICLOPYR

Herbicides were extracted from leaf material and analyzed as described in Materials and Methods. Three extracts were prepared for each treatment at each time interval and the results given constitute the mean ± one standard deviation. Full results are given in Appendix C

Herbicide Applied	Herbicide Detected	Days After Herbicide Applied				
		+1	+2	+3	+8	+14
		µg Herbicide Per Leaf				
2,4-D plus 2,4-DP	2,4-D	109±22	76±6	75±8	126±14	-
	2,4-DP	76±4	78±6	18±4	51±18	-
	Picloram	nd	nd	nd	nd	-
	Triclopyr	nd	nd	nd	nd	-
Picloram plus 2,4-D	2,4-D	110±21	133±22	121±20	125±15	39±3
	2,4-DP	nd	nd	nd	nd	nd
	Picloram	33±11	48±6	40±3	63±8	(34)*
	Triclopyr	nd	nd	nd	nd	nd
Triclopyr	2,4-D	nd	nd	nd	nd	nd
	2,4-DP	nd	nd	nd	nd	nd
	Picloram	nd	nd	nd	nd	nd
	Triclopyr	121±22	116±6	110±31	97±7	57±20

nd = not detected; - = no samples;

* = only one sample contained picloram and this is the value quoted.

The amounts of herbicide detected in 'washed' leaves of Carolina poplar and white ash trees which had been sprayed with the various herbicide treatments, are given in Tables III and IV respectively. The amounts of 2,4-D (applied in combination with 2,4-DP), 2,4-DP and triclopyr detected in Carolina poplar leaves sprayed with those herbicides declined by three days after herbicide application (Table III), however when 2,4-D was supplied in combination with picloram, there was no decline in the amount of either 2,4-D or picloram with time. The ratio of 2,4-D to picloram detected in 'washed' leaf extracts of Carolina poplar was approximately four to one, the same as in the original foliage spray solution.

In white ash, there was a decline in the amount of 2,4-D and 2,4-DP detected in 'washed' leaves between eight and seventeen days after application of a mixture of 2,4-D and 2,4-DP (Table IV). In trees sprayed with triclopyr there was a similar decline, which apparently occurred over the same period. However in trees sprayed with picloram plus 2,4-D, there was no obvious decline with time in the amount of either 2,4-D or picloram detected within the leaves.

In a comparison of the amount of the various herbicides expressed on a per g fresh weight basis in 'washed' leaves of Carolina poplar and white ash, the results were similar (see Appendices D and E). Such a comparison has to be made from the results expressed on a per g leaf fresh weight basis, because the fresh weights of typical Carolina poplar and white ash leaves are not the same. The similarity in the results with all the herbicides for 'washed' leaves of Carolina poplar and white ash suggests that purely on a whole leaf fresh weight basis, the concentration of each herbicide within the leaf does not differ between the two tree species. This does not necessarily mean that the herbicide concentrations within the leaf cells of the two species are similar, since individual herbicide molecules may reside within the leaf, without being within the individual leaf cells. It is conceivable that herbicide molecules may move inwards from the leaf surface after foliar-application but then remain ensconced in either the leaf cuticle or in the matrix of the plant cell walls. In previous reports such a distinction has rarely been made, perhaps because both removal of the leaf cuticle and cell walls, and extraction of the herbicide from them without disrupting the cell membrane, is technically difficult. It follows that any estimates of herbicide content of leaves obtained from surface washing experiments overestimate the amount of herbicide within the individual plant cells.

It is possible to estimate the percentage of retained herbicide that enters the leaves of Carolina poplar and white ash, by relating the amount of each herbicide detected in 'washed' leaves

TABLE III

HERBICIDE DETECTED IN 'WASHED' CAROLINA POPLAR LEAVES SPRAYED WITH 2,4-D PLUS 2,4-DP OR PICLORAM PLUS 2,4-D OR TRICLOPYR

Herbicides were extracted from leaf material and analyzed as described in Materials and Methods. Three extracts were prepared for each treatment at each time interval and the results given constitute the mean ± one standard deviation. Full results are given in Appendix D

Herbicide Applied	Herbicide Detected	Days After Herbicide Applied		
		+1	+3	+8
		µg Herbicide Per Leaf		
2,4-D plus 2,4-DP	2,4-D	123±4	57±4	70±16
	2,4-DP	52±17	16±5	19±7
	Picloram	nd	nd	nd
	Triclopyr	nd	nd	nd
Picloram plus 2,4-D	2,4-D	46±1	25±8	38±11
	2,4-DP	nd	nd	nd
	Picloram	12±1	6±2	9±2
	Triclopyr	nd	nd	nd
Triclopyr	2,4-D	nd	nd	nd
	2,4-DP	nd	nd	nd
	Picloram	nd	nd	nd
	Triclopyr	141±10	62±22	86±18

nd = not detected;

TABLE IV

HERBICIDE DETECTED IN 'WASHED' WHITE ASH LEAVES SPRAYED WITH 2,4-D PLUS 2,4-DP OR PICLORAM PLUS 2,4-D OR TRICLOPYR

Herbicides were extracted from leaf material and analyzed as described in Materials and Methods. Three extracts were prepared for each treatment at each time interval and the results given constitute the mean ± one standard deviation. Full results are given in Appendix E

Herbicide Applied	Herbicide Detected	Days After Herbicide Applied			
		+1	+3	+8	+17
		µg Herbicide Per Leaf			
2,4-D plus 2,4-DP	2,4-D	54±5	42±12	61±20	10±1
	2,4-DP	18±5	23±9	27±12	10±2
	Picloram	nd	nd	nd	nd
	Triclopyr	nd	nd	nd	nd
Picloram plus 2,4-D	2,4-D	38±21	7±2*	24±7	14±6*
	2,4-DP	nd	nd	nd	nd
	Picloram	11±5	3±3*	8±1	5±2*
	Triclopyr	nd	nd	nd	nd
Triclopyr	2,4-D	nd	nd	nd	nd
	2,4-DP	nd	nd	nd	nd
	Picloram	nd	nd	nd	nd
	Triclopyr	59±7	79±21	65±24	25±11

nd = not detected; * = 2 samples analyzed

to that detected in 'unwashed' leaves. These data are presented in Table V. In both Carolina poplar and white ash, approximately 50% of the 2,4-D (applied to the foliage in a mixture of 2,4-D and 2,4-DP) found in the foliage was within the leaf, and there was little change in this value up to eight days after initial herbicide application. Sharma and Vanden Born (1970) have also reported a penetration of aspen poplar (Populus tremuloides Michx) leaves by 50% of the ^{14}C-2,4-D applied, and this was achieved within 24 h of herbicide application. Eliasson and Hallmen (1973) reported that 67% of the applied 2,4-D had entered the leaves of aspen (Populus tremula) within 24 h of application. However Davis et al (1968b) found that only 1% of the applied 2,4,5-T was absorbed by the leaves of live oak (Quercus virginiana) after 48 h.

Twenty-four per cent or more of the 2,4-DP found in both Carolina poplar and white ash leaves appeared to reside within the leaves rather than on the surface, although the value of 128% for the proportion of 2,4-DP within the leaf cells of white ash after three days is obviously erroneous (see Table V). There have been no reports in the literature on the uptake of 2,4-DP by tree foliage.

When 2,4-D was applied to foliage of both Carolina poplar and white ash in combination with picloram, less 2,4-D appeared to enter the leaves (as a percentage of the total 2,4-D found in and on the leaves) than if 2,4-DP was applied in combination with the 2,4-D (Table V). The effect of one herbicide on the uptake of another when they are applied in combination is complex, in that Davis et al (1968a) found that more picloram was absorbed by leaves of honey mesquite (Prosopis juliflora var glandulosa) when picloram was supplied in combination with 2,4,5-T than if picloram was supplied alone. However less 2,4,5-T was absorbed by the plants if 2,4,5-T was supplied in combination with picloram than if 2,4,5-T was supplied alone. Not only was herbicide absorption into the leaves affected in this way, but downward transport via the plant stem was affected also, with the same positive effect of 2,4,5-T on picloram transport and negative effect of picloram on 2,4,5-T transport. Any stimulatory effect of 2,4-D on picloram entry into Carolina poplar and white ash leaves was not obvious, since the two herbicides were applied to the foliage in a ratio of 4 to 1, and in 'washed' leaves of either species, the ratio of 2,4-D to picloram detected was between 4 to 1 and 3 to 1 (Tables III and IV). What is strange however is that in 'unwashed' leaves of Carolina poplar and white ash, the ratio of 2,4-D to picloram was also invariably less than 4 to 1, yet since a large percentage of either herbicide detected was still on the surface of the leaves and presumably unchanged, if anything a more rigid adherence to a 4 to 1 ratio might have been expected in these

TABLE V

PROPORTION OF HERBICIDE DETECTED IN 'WASHED' LEAVES OF CAROLINA POPLAR AND WHITE ASH TO HERBICIDE DETECTED IN 'UNWASHED' LEAVES, AFTER APPLICATION OF 2,4-D PLUS 2,4-DP OR PICLORAM PLUS 2,4-D OR TRICLOPYR

This proportion constitutes the portion of total herbicide detected that is within the leaf (ie not on the surface).

Percentage of herbicide within the leaf

$$= \frac{\text{herbicide detected in 'washed' leaves (µg/leaf)}}{\text{herbicide detected in 'unwashed leaves' (µg/leaf)}} \times 100$$

Herbicide Applied	Herbicide Detected	Carolina Poplar			White Ash		
		Days After Herbicide Applied			Days After Herbicide Applied		
		+1	+3	+8	+1	+3	+8
		%	%	%	%	%	%
2,4-D plus 2,4-DP	2,4-D	59	40	43	50	56	48
	2,4-DP	59	80	36	24	128	53
Picloram plus 2,4-D	2,4-D	28	15	25	35	5	19
	Picloram	16	9	7	33	6	8
Triclopyr	Triclopyr	78	62	65	49	72	67

preparations (Tables I and II). It should be noted that combinations of herbicides are primarily used in the field to obtain a wider spectrum of effects, in that plants resistant to one herbicide of the combination may be sensitive to the other.

The small percentage of picloram detected in and on the leaves of Carolina poplar and white ash that is within the leaves is shown in Table V. In both species, the percentage of picloram within the leaves is less than 10% in samples collected three and eight days after herbicide application. In samples collected one day after application of herbicides to both species, the percentage of picloram within the leaf was greater than 10% for both species, and the subsequent decline might reflect translocation of the herbicide out of the leaf, although on the basis of picloram content per leaf, any decline in either species was not obvious (Tables III and IV).

Davis et al (1968b) found that foliar uptake of picloram varied greatly between different woody species. In live oak (Quercus virginiana Mill), and saw greenbriar (Smilax bona-nox L) approximately 2% of the applied picloram was absorbed after 48 h, whilst cottonwood (Populus deltoides Marsh), winged elm (Ulmus alata Michx) and honey mesquite (Prosopis glandulosa var glandulosa) absorbed 9%, 14% and 16% respectively. Bovey and Mayeux (1980) reported that 20% and 61% of the picloram applied to honey mesquite (Prosopis juliflora var glandulosa) was absorbed by three and ten days after application respectively. With such a wide variation in the proportion of picloram that is apparently taken up by different woody species it is difficult to draw any general conclusions. A partial explanation for the obvious discrepancies in these reports may lie in the slightly different experimental approaches used. Some workers have applied a known amount of ^{14}C-labelled herbicide to foliage and followed its movement with time, whereas others have applied non-labelled herbicide and followed its movement using classical analytic procedures such as gas-liquid chromatography. The efficiency with which herbicide on the surface of the leaf is removed will also influence any estimate of the herbicide within the leaf. The way in which results are expressed may also be important, in that most ^{14}C-labelled herbicide studies relate the amount of ^{14}C-label found within the leaf at any one time to the total amount of herbicide found (ie, on and within the leaf) at that time. Thus if the total amount of herbicide detected in and on the leaf falls with time, due to volatilization, translocation, herbicide metabolism or photolysis, and the amount within the leaf remains constant, then the percentage of herbicide within the leaf would appear to increase. Such a criticism has been conceded by Bovey and Mayeux (1980). In the present study the amounts of herbicide found within the leaf were related to the total amount of herbicide found at the same

time, however these latter values did not decrease with time, at least up to eight days after herbicide application, (see Tables I and II) and therefore the values for percentage uptake presented in Table V may remain valid.

The proportion of applied triclopyr that was detected within the leaves of Carolina poplar and white ash appeared to be consistently high, and did not decline with time (Table V). Bovey and Mayeux (1980) also reported that a large proportion of the triclopyr detected in honey mesquite (Prosopis juliflora var glandulosa) leaves was within the leaf, with 20% and 61% found within the leaves three and ten days after initial herbicide application respectively. However Bovey et al (1979) had reported earlier that huisache (Acacia farnesiana) leaves sprayed with triclopyr contained only approximately 10% of the total triclopyr detected up to 30 days after initial application.

3.2 Translocation of the Herbicides 2,4-D, 2,4-DP, Picloram and Triclopyr to the Roots of Carolina Poplar and White Ash After Application of 2,4-D plus 2,4-DP, Picloram plus 2,4-D or Triclopyr to the Foliage

The herbicides detected in the roots of Carolina poplar which had received a foliar spray of either 2,4-D plus 2,4-DP or triclopyr nine days previously are presented in Table VI. Root samples were collected from Carolina poplar trees which had received one or other of the herbicide treatments three, nine and fifteen days previously, and unless described in Table VI, none of the samples contained detectable herbicide. All three root samples from trees sprayed nine days previously with 2,4-D plus 2,4-DP contained 2,4-D, while all three root samples from trees sprayed with triclopyr contained triclopyr. The detection limit for herbicides in root samples was approximately 2 µg/g root fresh weight, however this varied according to the fresh weight of the sample (for discussion see Materials and Methods), and thus some of the other root samples from trees sprayed with herbicide either three, nine or fifteen days previously may have contained similar concentrations of herbicides as those given in Table VI. The fact that neither 2,4-DP nor picloram were detected in any of the Carolina poplar root samples may be correlated with the finding that in leaf samples levels of 2,4-DP detected were generally lower than those for 2,4-D (in plants sprayed with 2,4-D plus 2,4-DP) and hence the levels of 2,4-DP in the roots were probably below the detection limit. In trees sprayed with 2,4-D plus picloram, picloram constituted only 20% by weight of this mixture, and since very small amounts of 2,4-D were detected in the roots of some of these trees, it would have been unexpected to find detectable amounts of picloram.

TABLE VI

HERBICIDE DETECTED IN CAROLINA POPLAR ROOTS NINE DAYS AFTER APPLICATION TO THE FOLIAGE OF 2,4-D PLUS 2,4-DP OR TRICLOPYR

Same conditions as outlined in legend to Table 1 except root material was extracted and all the sample values are quoted here.

Results are expressed as µg herbicide per g root fresh weight.

Herbicide Detected	Herbicide Applied							
	2,4-D plus 2,4-DP				Triclopyr			
	Sample Number			Mean Value ± One Standard Deviation	Sample Number			Mean Value ± One Standard Deviation
	1	2	3		1	2	3	
2,4-D	2.8	1.9	2.1	2.3±0.5	nd	nd	nd	-
2,4-DP	nd	nd	nd	-	nd	nd	nd	-
Picloram	nd	nd	nd	-	nd	nd	nd	-
Triclopyr	nd	nd	nd	-	3.1	2.5	3.5	3.0±0.5

nd = not detected

N.B. No herbicide was detected in root extracts from plants sprayed with picloram plus 2,4-D nine days previously, or in root extracts from plants sprayed with 2,4-D plus 2,4-DP or picloram plus 2,4-D or triclopyr three and fifteen days previously. Fifteen days after all herbicide treatments all foliage was dead; defoliation occurred later.

In roots collected from white ash sprayed with either of the three herbicide treatments three, nine, fifteen and twenty-four days previously, only one sample was found to contain herbicide. One tree sprayed with 2,4-D plus picloram three days previously, was found to contain 1.1 µg picloram per g fresh weight, but no detectable 2,4-D. In the light of the disproportionate amount of picloram in the original foliar spray solution, this was unexpected. It might be the result of more efficient translocation of picloram (than 2,4-D) from the foliage to the roots or a greater rate of 2,4-D degradation or complexation. Although no detectable triclopyr was found in the roots of white ash trees sprayed with the herbicide, the rate of respiration of the roots from these trees appeared to be inhibited (Ontario Hydro Research Division Report No 83-88-K), as was the case with roots from white ash sprayed with picloram plus 2,4-D and roots from Carolina poplar sprayed with either of the three herbicide treatments. Root respiration may have been inhibited either by the herbicide directly (in some cases the level of herbicide may have been below the detectable limit) by a phytotoxic herbicide metabolite or as a secondary effect as the result of a shortage of respiratory substrate, for example.

The amounts of herbicide detected in the roots of Carolina poplar and white ash trees described above are similar to those published previously for woody species. The herbicide contents of the roots of three woody species to which herbicide had been applied as a foliar-spray are given in Table VII. Triclopyr and picloram were applied to the foliage of honey mesquite (Prosopis juliflora var glandulosa) at a rate of 1.1 kg/ha, both were effective in killing the plant (Bovey and Mayeux, 1980), yet the time of appearance of the respective herbicides in the roots, and the concentrations of herbicides found were slightly different. With time there appeared to be a reduction in the concentration of both triclopyr and picloram found in the roots. Although the same amount of picloram or 2,4,5-T was applied to mesquite (Prosopis glandulosa var glandulosa), there was nearly an order of magnitude difference in the concentration of the two herbicides found in the roots (Davis et al, 1968c). In huisache (Acacia farnesiana) the same amount of picloram or triclopyr was applied (1.12 kg/ha) and the same amount of each herbicide found in the roots (Bovey et al, 1979). This last study also found that triclopyr was accumulated in the stem and leaf tissues to a smaller extent than picloram, which the authors concluded might indicate metabolic breakdown of triclopyr or less efficient translocation of triclopyr than of picloram.

Are the small amounts of foliar-applied herbicide that end up in the roots of these woody plants enough to kill the roots and the plant as a whole? Unfortunately this question was not addressed directly in any of the studies listed in Table VII. Bovey and

TABLE VII

PARTIAL LIST OF REPORTS ON THE HERBICIDE CONTENT OF ROOTS OF WOODY SPECIES IN WHICH TRICLOPYR, PICLORAM OR 2,4,5-T HAD BEEN APPLIED TO THE FOLIAGE

Plant Species	Herbicide Detected	Amount in Roots µg/g fresh weight	Days After Herbicide Application to Foliage	Reference
Honey mesquite (Prosopis juliflora var glandulosa)	Triclopyr	1.0	3	Bovey & Mayeux (1980)
	"	0.8	10	
	"	0.3	30	
	Picloram	0.7	3	
	"	2.1	10	
	"	0.3	30	
Mesquite (P. glandulosa var glandulosa)	Picloram	0.55	4	Davis et al (1968c)
	2,4,5-T	0.07	4	
Huisache (Acacia farnesiana)*	Triclopyr	0.2	3	Bovey et al (1979)
	"	0.3	10	
	"	0.2	30	
	Picloram	0.2	3	
	"	0.3	10	
	"	0.2	30	

* Values calculated from data given in ppm.

Mayeux (1980) reported that 12 weeks after foliar-application of either picloram or triclopyr, all honey mesquite topgrowth appeared dead, however no data were presented on root damage. In their earlier study Bovey et al (1979) did not equate root damage and herbicide content. While foliar-application of picloram caused complete defoliation of huisache within six weeks, triclopyr caused only 68% defoliation. Picloram was more destructive that triclopyr to root tissue. Thirty days after herbicide application, lateral root initiation was stimulated by most triclopyr treatments (soil and foliar-application). Foliar-application of picloram produced root injury ratings of six (on a subjective scale of zero to ten) whilst triclopyr produced a rating of less than one. Thus concentrations of picloram within the root of approximately 0.2 µg/g root fresh weight caused visible root damage, whilst similar concentrations of triclopyr were almost without effect.

In general it may not be possible to equate topgrowth death in tree species with complete plant death, in that the roots of some species if not killed, will sucker in the following year. Long-term experiments should be conducted where the herbicide content of the roots of tree species to which herbicide has been applied in various ways is correlated with suckering and sprouting in subsequent growing seasons.

Although it is useful to know the concentration of herbicide that is found in the roots after foliar-application (on a fresh weight basis), it remains important to know the percentage of applied herbicide that reaches the roots, since this would describe the efficiency of the application process given that killing the root is the primary objective in woody plant control. In the present study it was only possible to make an estimate of the percentage of herbicide that reached the roots of Carolina poplar and white ash, since it was not known how much herbicide was applied initially ('to leaf drip'). One day after herbicide application, leaves of Carolina poplar typically contained (on and in the leaf) approximately 300 µg of 2,4-D or triclopyr per g fresh weight (from Appendix B) and thus with between 2 and 3 µg of 2,4-D or triclopyr per g fresh weight found in the root (Table VI), this represented a percentage recovery of less than one. It was assumed that the total fresh weight of all the leaves on a tree was the same as the fresh weight of all the roots, however the former may in fact be higher, and if so, the percentage of applied herbicide that reached the root would be lower still. For white ash the percentage of picloram that reached the root was also less than one. These approximations are similar to those reported previously, in that 1% of the 2,4-D applied to the foliage of aspen (Populus tremula) was found in the roots nine days after application (Eliasson and Hallmen, 1973). Leonard et al (1966)

reported that 0.4% of the 2,4,5-T applied to white ash had reached the roots after 30 days, while none had reached the roots of red maple (Acer rubrum L). Although such values reflect the efficiency (or inefficiency!) of the method of herbicide application in getting chemical to the plant root, they do not reveal the concentration of that chemical in the root, and it is this which may reveal the inherent susceptibility or resistance of any particular species to herbicide-induced root death. Mitchell and Stephenson (1973) found that the concentration of picloram in the roots of white ash was higher than that in the roots of red maple (Acer rubrum L) yet the former species was more resistant to the herbicide. This prompted these authors to conclude that since white ash appears to be more tolerant than red maple (Acer rubrum L) to picloram at the cellular level, then any increase in the efficiency of herbicide uptake and translocation may not increase the effectiveness of picloram for the control of white ash.

As has been alluded to on several occasions in this report, there are distinct problems inherent in this type of herbicide study. Herbicide extraction and estimation procedures are not as efficient as they might be. The problem of expressing herbicide content on the basis of plant fresh weight should be avoided and it would be better to express it as a function of leaf area and this would allow an easier comparison to be made between plant species with leaves of different shapes and areas.

CONCLUSIONS

After taking into account the variability in the data obtained, the following tentative conclusions can be drawn from the experiments described in this study.

1. One day after application of the herbicides triclopyr, picloram, 2,4-DP and 2,4-D (applied in combination with either 2,4-DP or picloram) to the foliage of Carolina poplar and white ash, leaves of white ash had retained slightly more 2,4-D, 2,4-DP, picloram and triclopyr either in or on the leaves, than leaves of Carolina poplar, on a leaf fresh weight basis.

2. Of the total herbicide detected within and on the surface of leaves of Carolina poplar and white ash, a minimum of 30% of the 2,4-DP, 2,4-D (applied with 2,4-DP) and triclopyr appeared to reside within the leaves one day after herbicide application. Up to eight days after application there was little consistent change in the percentage of 2,4-DP, 2,4-D (applied with 2,4-DP) and triclopyr detected

within the leaves of either woody species. However on a weight of herbicide detected per leaf basis, the amounts of all three herbicides found within the leaves of Carolina poplar and white ash appeared to decline by the third day after application to the former woody species and by the seventeenth day after application to the latter.

3. There was little translocation of any of the applied herbicides from the foliage of Carolina poplar and white ash to the roots, since only small amounts of triclopyr and 2,4-D (when applied in combination with 2,4-DP), on a fresh weight basis, were detected in root tissue of Carolina poplar, and a small amount of picloram was the only herbicide detected in the roots of white ash.

4. There was no correlation between either a lack of retention of any of the applied herbicides on foliage, or a lack of uptake of the retained herbicide into the leaf, with the overall herbicide-resistance of white ash. However, although both triclopyr and 2,4-D were detected in the roots of Carolina poplar (a herbicide-sensitive woody species) neither herbicide was detected in the roots of white ash (a herbicide-resistant woody species).

Approved:

D. Harrison
Manager
Chemical Research Dept

GPA:lg

Submitted:

G.P. Arron
Biologist
Biological Research Section

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APPENDIX A

HERBICIDE COMPOSITION

2,4-D Plus 2,4-DP

The mixture of 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4-DP (2,4-diclorophenoxypropionic acid) was obtained from Union Carbide Agricultural Products, batch number S77107 under the trade name Desormone 700. The herbicide mixture contained the butoxy-ethanol ester derivatives of 2,4-D (350 g/L acid equivalent) and 2,4-DP (350 g/L acid equivalent). The stock solution of herbicide was diluted 100 times with tap water and sprayed on the tree foliage as described in Materials and Methods.

Picloram Plus 2,4-D

The mixture of picloram (4-amino 3,5,6-trichloropicolinic acid, triisopropanolamine salt) and 2,4-D (2,4-dichlorophenoxyacetic acid, triisopropanolamine salt) under the trade name Tordon 101 was obtained from Dow Chemical of Canada Limited, via various intermediaries (lot number unknown). The stock solution contained 60 g/L acid equivalent of picloram and 240 g/L acid equivalent of 2,4-D. This stock solution was diluted 100 times with tap water before application to the tree foliage.

Triclopyr

The stock solution of triclopyr {[(3,4,5-trichloro-2-pyridinyl) oxy] acetic acid} was obtained from Dow Chemical of Canada Limited under the trade name Garlon 4 (or Dowco 233) (Lot number unknown). This herbicide is registered for use in the USA but not in Canada (other than for research purposes). Garlon 4 is an oil soluble, water emulsifiable ethylene glycol butyl ether ester formulation. The stock solution of Garlon 4 contained 480 g/L acid equivalent of triclopyr. This stock solution was diluted 133 times with tap water prior to application to the tree foliage.

APPENDIX B

HERBICIDE DETECTED IN 'UNWASHED' LEAVES OF CAROLINA POPLAR SPRAYED WITH 2,4-D PLUS 2,4-DP, PICLORAM PLUS 2,4-D OR TRICLOPYR

Herbicides were extracted from leaf material in triplicate at each time interval and analyzed as described in Materials and Methods. Results are presented for each sample together with the mean ± one standard deviation, and are expressed as herbicide in μg per leaf or per g leaf fresh weight.

nd = not detected; nd(3) = not detected in 3 samples; - = no sample analyzed;
* mean of two samples (out of three) which contained detectable herbicide, foliage had probably been contaminated in greenhouse (for discussion of cross-contamination see Materials and Methods)

Applied Herbicide	Detected Herbicide	Days After Herbicide Applied									
		μg per leaf									
		+1		+2		+3		+8		+14	
		Sample	Mean	Sample	Mean	Sample	Mean	Sample	Mean	Sample	Mean
2,4-D plus 2,4-DP	2,4-D	205 235 190	210 ±23	152 189 146	162 ±23	154 119 150	141 ±19	144 183 -	164 ±28	- - -	
	2,4-DP	101 85 79	88 ±11	41 54 51	49 ±7	26 12 21	20 ±7	46 60 -	53 ±10	- - -	
	Picloram	nd(3)		nd(3)		nd(3)		nd(2)		-	
	Triclopyr	nd(3)		nd(3)		19 33 nd	(26±10)*	nd(2) -		- - -	
Picloram plus 2,4-D	2,4-D	182 147 171	167 ±18	190 190 197	192 ±4	152 183 159	165 ±16	258 212 291	254 ±40	337 201 265	268 ±68
	2,4-DP	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
	Picloram	76 71 74	74 ±3	69 67 70	69 ±2	57 70 69	65 ±7	133 115 143	130 ±14	151 51 132	111 ±53
	Triclopyr	nd(3)		nd(3)		23 45 33	34 ±11	nd(3)		nd(3)	
Triclopyr	2,4-D	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
	2,4-DP	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
	Picloram	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
	Triclopyr	179 217 148	181 ±35	202 157 162	174 ±25	90 108 102	100 ±9	168 95 137	133 ±37	92 127 193	137 ±51

Applied Herbicide	Detected Herbicide	Days After Herbicide Applied									
		μg per g leaf Fresh Weight									
		+1		+2		+3		+8		+14	
		Sample	Mean	Sample	Mean	Sample	Mean	Sample	Mean	Sample	Mean
2,4-D plus 2,4-DP	2,4-D	267 306 247	273 ±30	209 261 201	224 ±33	120 93 117	110 ±15	205 262 -	234 ±40	- - -	
	2,4-DP	131 110 103	115 ±15	57 74 70	67 ±9	20 9 16	15 ±6	66 86 -	76 ±14	- - -	
	Picloram	nd(3)		nd(3)		nd(3)		nd(2)		-	
	Triclopyr	nd(3)		nd(3)		15 26 nd	(21±8)*	nd(2)		- - -	
Picloram plus 2,4-D	2,4-D	258 208 243	236 ±26	259 259 269	262 ±5	133 161 140	145 ±15	540 444 609	531 ±83	775 463 552	597 ±161
	2,4-DP	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
	Picloram	108 100 104	104 ±4	92 94 95	94 ±2	50 62 61	57 ±7	279 241 299	273 ±29	347 117 303	256 ±122
	Triclopyr	nd(3)		nd(3)		20 40 29	30 ±10	nd(3)		nd(3)	
Triclopyr	2,4-D	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
	2,4-DP	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
	Picloram	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
	Triclopyr	293 355 242	297 ±56	288 224 231	248 ±35	78 94 88	87 ±8	272 155 222	216 ±59	227 313 476	339 ±126

APPENDIX C

HERBICIDE DETECTED IN 'UNWASHED' LEAVES OF WHITE ASH SPRAYED WITH 2,4-D PLUS 2,4-DP, PICLORAM PLUS 2,4-D OR TRICLOPYR

Herbicides were extracted from leaf material in triplicate at each time interval and analyzed as described in Materials and Methods. Results are presented for each sample together with the mean ± one standard deviation, and are expressed as herbicide in μg per leaf or per g leaf fresh weight.

nd(3) = not detected in 3 samples; - = no sample analyzed; * only one sample (of three) contained herbicide, and since detection limits vary from sample to sample (for discussion of detection limits see Materials and Methods) a mean could not be calculated.

Applied Herbicide	Detected Herbicide	Days After Herbicide Applied									
		μg per leaf									
		+1		+2		+3		+8		+14	
		Sample	Mean	Sample	Mean	Sample	Mean	Sample	Mean	Sample	Mean
2,4-D plus 2,4-DP	2,4-D	89 105 133	109 ±22	80 70 87	77 ±6	79 67 57	75 ±8	111 129 139	126 ±14	- - -	
	2,4-DP	78 78 71	76 ±4	78 72 86	78 ±6	22 16 15	18 ±4	71 40 41	51 ±18	- - -	
	Picloram	nd(3)		nd(3)		nd(3)		nd(3)		-	
	Triclopyr	nd(3)		nd(3)		nd(3)		nd(3)		-	
Picloram plus 2,4-D	2,4-D	131 90 109	110 ±21	121 119 158	133 ±22	131 134 98	121 ±20	129 108 138	125 ±15	42 40 36	39 ±3
	2,4-DP	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
	Picloram	41 21 38	33 ±11	54 43 48	48 ±6	41 42 36	40 ±3	72 59 58	63 ±8	nd 34 nd	(34)*
	Triclopyr	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
Triclopyr	2,4-D	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
	2,4-DP	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
	Picloram	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
	Triclopyr	141 97 124	121 ±22	110 115 122	116 ±6	88 96 146	110 ±31	103 90 97	97 ±7	64 72 34	57 ±20

Applied Herbicide	Detected Herbicide	Days After Herbicide Applied									
		μg per g Leaf Fresh Weight									
		+1		+2		+3		+8		+14	
		Sample	Mean	Sample	Mean	Sample	Mean	Sample	Mean	Sample	Mean
2,4-D plus 2,4-DP	2,4-D	362 427 542	444 ±91	324 283 354	321 ±36	162 136 116	138 ±23	360 301 325	295 ±33	- - -	
	2,4-DP	319 320 289	309 ±18	317 292 349	320 ±24	45 32 30	36 ±8	167 93 96	119 ±42	- - -	
	Picloram	nd(3)		nd(3)		nd(3)		nd(3)		-	
	Triclopyr	nd(3)		nd(3)		nd(3)		nd(3)		-	
Picloram plus 2,4-D	2,4-D	570 394 478	481 ±88	469 462	516 ±87	339 346 252	313 ±52	259 217 287	250 ±30	153 146 133	144 ±10
	2,4-DP	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
	Picloram	178 91 164	144 ±47	211 166 187	188 ±23	107 109 92	103 ±9	144 118 115	126 ±16	nd 125 nd	(125)*
	Triclopyr	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
Triclopyr	2,4-D	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
	2,4-DP	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
	Picloram	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
	Triclopyr	528 363 466	452 ±84	479 501 534	505 ±28	175 190 291	207 ±43	346 302 324	324 ±22	282 317 151	250 ±68

APPENDIX D

HERBICIDE DETECTED IN 'WASHED' LEAVES OF CAROLINA POPLAR SPRAYED WITH 2,4-D PLUS 2,4-DP, PICLORAM PLUS 2,4-D OR TRICLOPYR

Same conditions as described in legend to Appendix B

nd(3) = not detected in 3 samples analyzed

Applied Herbicide	Detected Herbicide	Days After Herbicide Applied											
		µg per leaf						µg per g Leaf Fresh Weight					
		+1		+3		+8		+1		+3		+8	
		Sample	Mean	Sample	Mean	Sample	Mean	Sample	Mean	Sample	Mean	Sample	Mean
2,4-D plus 2,4-DP	2,4-D	126 118 124	123 ±4	71 58 43	57 ±14	52 76 83	70 ±16	166 167 147	160 ±11	53 30 47	43 ±12	93 92 118	101 ±15
	2,4-DP	45 71 39	52 ±17	14 21 12	16 ±5	13 17 26	29 ±7	59 101 46	69 ±29	10 11 13	11 ±2	23 21 36	27 ±8
	Picloram	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
	Triclopyr	nd(3)		nd(3)		nd(3)		nd(3		nd(3)		nd(3)	
Picloram plus 2,4-D	2,4-D	45 47 47	46 ±1	22 20 34	25 ±8	50 36 29	38 ±11	57 63 79	66 ±11	23 20 21	21 ±2	67 89 82	79 ±11
	2,4-DP	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
	Picloram	13 11 13	12 ±1	5 5 8	6 ±2	12 8 8	9 ±2	16 15 22	18 ±4	6 4 5	5 ±1	16 19 21	19 ±3
	Triclopyr	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
Triclopyr	2,4-D	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
	2,4-DP	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
	Picloram	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
	Triclopyr	151 141 132	141 ±10	87 46 52	62 ±22	66 98 95	86 ±18	213 232 255	233 ±21	324 130 189	214 ±99	153 103 172	143 ±36

APPENDIX E

HERBICIDE DETECTED IN 'WASHED' LEAVES OF WHITE ASH SPRAYED WITH 2,4-D PLUS 2,4-DP, PICLORAM PLUS 2,4-D OR TRICLOPYR

Same conditions as described in legend to Appendix B

nd(3) = not detected in 3 samples analyzed; - = no sample analyzed

Applied Herbicide	Detected Herbicide	Days After Herbicide Applied															
		μg per leaf								μg per g Leaf Fresh Weight							
		+1		+3		+8		+17		+1		+3		+8		+17	
		Sample	Mean	Sample	Mean	Sample	Mean	Sample	Mean	Sample	Mean	Sample	Mean	Sample	Mean	Sample	Mean
2,4-D plus 2,4-DP	2,4-D	59 54 50	54 ±5	32 55 40	42 ±12	52 47 84	61 ±20	10 9 -	10 ±1	224 233 207	221 ±13	86 82 79	82 ±4	149 116 155	140 ±21	55 49 -	52 ±4
	2,4-DP	14 16 24	18 ±5	17 33 18	23 ±9	15 27 38	27 ±12	11 8 -	10 ±2	52 70 98	73 ±23	44 49 36	43 ±7	44 65 70	60 ±14	56 45 -	51 ±8
	Picloram	nd(3)		nd(3)		nd(3)		nd(2)		nd(3)		nd(3)		nd(3)		nd(2)	
	Triclopyr	nd(3)		nd(3)		nd(3)		nd(2)		nd(3)		nd(3)		nd(3)		nd(2)	
Picloram plus 2,4-D	2,4-D	24 63 28	38 ±21	5 8 -	7 ±2	19 32 22	24 ±7	9 18 -		97 258 142	166 ±83	22 14 -	18 ±6	48 49 48	48 ±1	57 32 -	45 ±18
	2,4-DP	nd(3)		nd(2)		nd(3)		nd(2)		nd(3)		nd(2)		nd(3)		nd(2)	
	Picloram	8 17 9	11 ±5	1 5 -	3 ±3	7 9 7	8 ±1	3 6 -	5 ±2	31 70 44	48 ±20	5 7 -	6 ±1	17 14 15	15 ±2	10 18 -	14 ±6
	Triclopyr	nd(3)		nd(2)		nd(3)		nd(2)		nd(3)		nd(2)		nd(3)		nd(2)	
Triclopyr	2,4-D	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
	2,4-DP	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
	Picloram	nd(3)		nd(3)		nd(3)		nd(3)		nd(3)		nd(3)		nd(3)		nd(3)	
	Triclopyr	67 55 54	59 ±7	95 55 87	79 ±21	38 70 86	65 ±24	12 32 30	25 ±11	231 237 196	221 ±22	173 127 161	154 ±24	205 180 232	206 ±26	152 281 238	224 ±66

POOR

QUALITY

ORIGINAL